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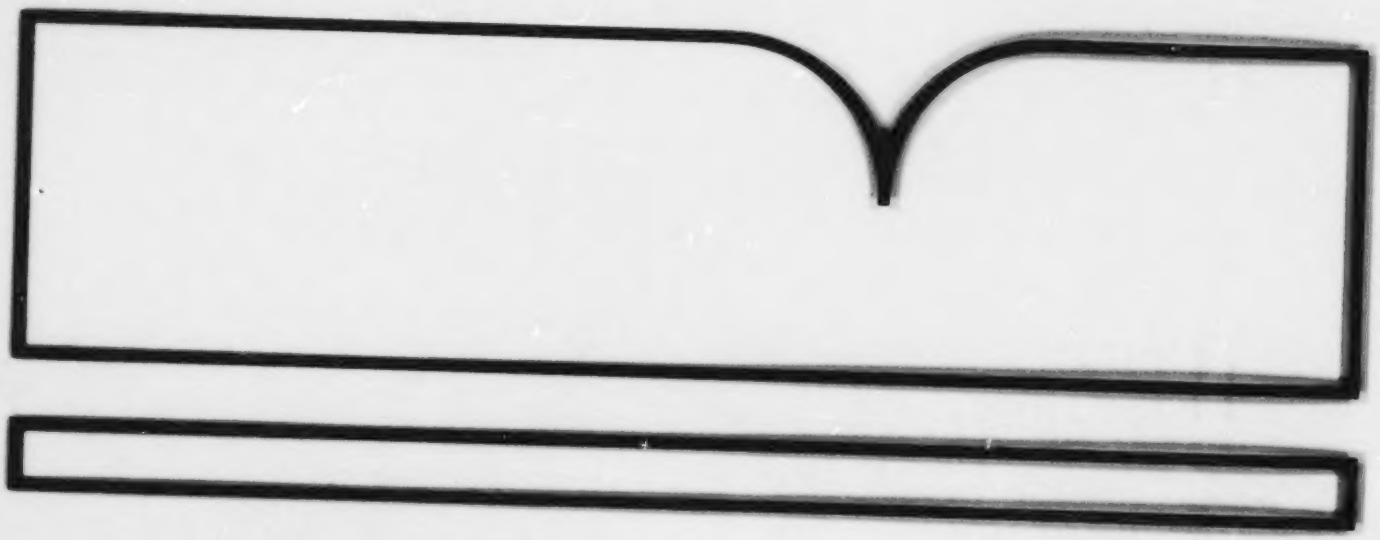

U.S. Department of Commerce
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HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

QUALITATIVE NATURE OF THE RESIDUE:

PLANT METABOLISM

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Plant metabolism studies are designed to characterize the qualitative nature of the total terminal residue in plants. Specifically, these studies should provide an estimate of total residues in the treated crop, identify the major components of the total terminal residue thus indicating the components to be looked for in residue quantification studies, indicate the distribution of residues in the treated crop, and show the efficiency of extraction procedures for various components of the residue. This SEP describes in a detailed, sequential stepwise manner how EPA reviewers evaluate plant metabolism studies, and what type of information is taken into consideration in the review process.

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STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.

Anne L. Barton, Acting Director
Hazard Evaluation Division

RESIDUE CHEMISTRY BRANCH

STANDARD EVALUATION PROCEDURE

Qualitative Nature of the Residue: Plant Metabolism

I. INTRODUCTION

A. Purpose of the Standard Evaluation Procedure

This Standard Evaluation Procedure (SEP) is designed to aid Residue Chemistry Branch (RCB) data reviewers in their evaluations of plant metabolism studies which qualitatively determine pesticide residues and their transformation in plants. This particular SEP document addresses plant metabolism studies only; the topic of animal metabolism studies will be addressed in a separate SEP document.

B. Background Information

Plant metabolism studies provide residue chemistry data on the qualitative nature of the residue in plants and are required by 40 CFR 158.125 to support the registration of any pesticide intended for use on a food or feed crop under the amended Federal Insecticide, Fungicide, and Rodenticide Act.

The Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry which are nonregulatory companions to 40 CFR Part 158 describe in Section 171-4(a)(1) and (2) protocols which may be used to perform plant metabolism studies. Section 171-4(a)(2) of these Guidelines states in part that: "A metabolism study must be submitted for each type of plant for which use is proposed and generally one metabolism study will be required for each of the crop groups defined in 40 CFR 180.34(f), except for herbs and spices. If the results of three metabolism studies on dissimilar crops indicate a similar metabolic route in the three crops, then additional metabolism studies will not be required."

C. Objective of Plant Metabolism Studies

Plant metabolism studies are designed to characterize the qualitative nature of the total terminal residue in plants and answer the question, what is the chemical residue? Specifically these studies should (1) provide an estimate of total residues in the treated crops; (2) identify the major components of the total terminal residue, thus indicating the components to be looked for in residue quantification studies; (3) indicate the distribution of residues, e.g., whether translocation occurs, or whether the residues are entirely surface residues; and (4) show the efficiency of extraction procedures for various components of the

residue. Requirements of these studies should be extended to also include characterization of residues in all plant parts used for food or feed. For example, if residues of concern (i.e., of toxicological significance) are found in animal feed items or in plant parts which can be converted to processed commodities, then animal metabolism and processed commodity studies respectively will be required.

In the final analysis, plant metabolism studies should answer the following question: What is the qualitative nature of the total terminal residue that will result in or on the raw food or feed commodity as a result of application of the pesticide formulated product according to the proposed label directions for use? Plant metabolism studies by characterizing the qualitative nature of the total terminal residue thereby facilitates a chemical definition of the "total toxic residue".

The term "total toxic residue" is defined as the sum of the parent pesticide and its degradation products, metabolites (free or bound), and impurities that are considered to be of toxicological significance, and therefore warrant regulation.

II. INFORMATION TO BE SUPPLIED

The petitioner/registrant's report on plant metabolism studies should include all information necessary to provide a complete and accurate description of treatments and procedures: radiolabeling techniques to include rate, method, and time of radiolabel application in relation to the development and growth cycle of the treated raw agricultural commodity (RAC); extraction, fractionation, and characterization techniques employed for the identification of residue components whether free or bound at each sampling interval; definition of total terminal residues, to include data for all major components of the total terminal residue reflecting their distribution within the RAC (including animal feed items or plant parts which can be converted to processed commodities) expressed as both percentage of the total recovered plant radioactivity and quantity (ppm) found, at time of harvest or when utilized for animal feed; reporting of data to include a delineation, preferably in a flowsheet, of possible routes of degradation or metabolism in plants,[and when enforcement analytical methodology has been developed, validation of these methods with radiolabeled samples derived from the plant metabolism study, accompanied by a statement made as to their capability to determine all components of the total toxic residue, whether free or bound/conjugated in the RAC] and quality control measures/ precautions taken to ensure validity of all aspects of the plant metabolism study.

[Note: If the specified bracketed statement/ information is provided elsewhere within the overall data submission package it need not be reiterated in the plant metabolism study but referenced as to location in the data submission package.]

III. THE DATA EVALUATION PROCESS

A. Determine the Need for the Study

The reviewer should initially determine whether the study is required under 40 CFR Part 158. Normally, a plant metabolism study is required to support the registration of any pesticide intended for use on a food or feed crop and accordingly must be submitted for each type of plant for which use is proposed. Exceptions to the latter requirement are clearly delineated in Section 171-4(a)(2) of the Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry.

B. Read the Report and Identify Data Gaps

Using the Data Reporting Guidelines on Nature of the Residue: Plants, the reviewer should look for data gaps-omissions in the information supplied by the petitioner/registrant in his report. These should be duly noted in the reviewer's report, and a judgment made as to which are considered significant enough to adversely affect the review process. Those so identified should be communicated back to the petitioner/registrant by the Product Manager for corrective action.

C. Assess the Appropriateness and Adequacy of the Data

The data reviewer then considers the appropriateness (i.e., the relevancy of both the test crop selected and the submitted radiolabel protocol to the intended use pattern of the pesticide) of the study including the adequacy of all supporting data/information that have been supplied. Appendix 1 of this document discusses specific aspects of testing parameters that warrant consideration.

As an adjunct to these, the reviewer should draw upon the technical guidance in the reviewer aids materials that are available to him, such as the Residue Chemistry Guidelines (a.k.a. Subdivision O of the Pesticide Assessment Guidelines). A listing of some suggested source materials is located in Appendix 2 to this document.

Any perceived deficiencies in the data/information supplied should be identified and explained, with a statement as to what steps should be taken to resolve the deficiencies, so that this information can be relayed back to the petitioner/registrant by the Product Manager for appropriate action.

D. Determine the Need for Deference/Reference to Other HED Branches

In considering the appropriateness and adequacy of the data/information that have been supplied in support of a plant metabolism study, the data reviewer must also determine if deference/reference(s) to other HED Branch(es) is/are germane to reaching that decision.

In the case of plant metabolism studies, specific deference/reference(s) may be made to Toxicology Branch (TB) to elicit their opinion regarding the toxicological significance of all characterized/identified components, whether free or bound, of the total terminal residue in the subject crop (including processed fractions derived therefrom) at time of harvest or when utilized for animal feed.

Situations in which deferences to TB are appropriate have been described in the SEP on Animal Metabolism (See Deferrals paragraph under WRITING A REVIEW of that document).

In the interim period until the response(s) to such deference/reference(s) is/are received, final judgment by the data reviewer on the issues(s) in questions should be withheld.

E. Make a Regulatory Recommendation

If the submitted plant metabolism study adequately defines the qualitative nature of the total terminal residue in the subject crop, then the data reviewer should decide which components of this residue are in need of regulation, i.e., those considered as comprising the total toxic residue in plants, in need of enforcement methodology and furthermore to be included in the tolerance expression. For a detailed discussion of helpful criteria for determining whether a metabolite should be included in the tolerance expression see Item 10, Expression of a Tolerance under Data Requirements in the SEP on Animal Metabolism, including the flowsheet diagram included as Appendix I to that document. These recommendations on the part of the data reviewer may require the assistance of TB through their responses to RCB's deference/ reference(s) described in D, above.

IV. REVIEWER AIDS

There are a large number and variety of source materials that are available to assist the data reviewer in the evaluation process. A listing of some of the more useful references that reside within the Branch are provided in Appendix 2 to this document.

APPENDIX 1

Major points to consider in evaluating plant metabolism data include the following:

A. Overall Protocol Considerations:

1. Conduct of the Test

Has the petitioner/registrant adequately described the overall testing environment including environmental controls imposed, if any, during the conduct of the plant metabolism study (i.e.,environmentally uncontrolled outdoor test plot, semi-controlled greenhouse environment, or completely controlled and monitored growth chamber)?

The reviewer should be aware that if any of the reported environmental conditions, including testing media selected, are atypical or at great variance with the expected cultural practices or environments under which the selected test crop is grown, then the resultant metabolism data obtained may be suspect and therefore not reflective of the proposed use.

2. The Test Crop

Has the test crop been adequately described in the submitted metabolism study? If so, is it the same crop for which use is proposed? If not, consult Section 171-4(a)(2) of the Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry and 40 CFR 180.34(f) regarding criteria for translating radiolabeled metabolism data between crop species.

Were all specific crop parts harvested and subjected to ^{14}C residue analysis for a determination of the total terminal residue clearly identified? Do they conform to Ref. Table II of Subdivision O, Residue Chemistry Guidelines?

Was the treated test crop grown to harvest (maturity) and/or to a developmental (immature) stage when normally utilized for animal feed, and are all the important dates relative to the growing and harvesting of the test crop reported?

Is there any other unpublished or published information known to us regarding the metabolism of this pesticide on the subject or botanically-related crop that must be taken into consideration?

3. The Radiolabled Pesticide

Was the original source of radiolabel reported including the purity and specific activity of the prepared radiolabled pesticide product in Curies/mole or disintegrations per minute per gram (dpm/g)?

If radiolabels other than ^{14}C were employed (i.e., ^{3}H, ^{32}P, and ^{35}S), was rationale given for their use?

Was radiolabeling in a potentially labile side chain or other potentially unstable position in the molecule avoided?

Was pesticide chemical labeled in a strategically important place in the molecule (i.e., preferably in ring nuclei if present) or if not present, at secondary or tertiary branching locations in the molecule?

Was multiple radiolabeling attempted for complex pesticide molecules containing multiple ring structures that may be cleaved or readily separated as a result of metabolism?

4. Application of the Pesticide

Overall, did the plant metabolism protocol reflect or mimic, to the extent possible, the intended use pattern (proposed labeling) of the pesticide on the test crop (i.e., preplant soil incorporated, over-the-top postemergent foliar application, bait application, lay-by application, etc.)?

In what formulation (i.e., solvent, carrier, bait, adjuvant or other matrix) was the radiolabeled pesticide applied, and by what means (i.e., hand sprayer, topical application, soil injection, etc.)?

Did the total rate of application to the test crop reflect at least the maximum allowable rate depending on the chemical and the test conditions employed? Exaggerated doses may be needed to ensure sufficient radioactivity for the purposes of residue identification/characterization.

Were the timing and frequency of radiolabel applications reported and were treated plants harvested at maturity or when normally utilized for animal feed?

Was an explanation or rationale provided by the petitioner/registrant for any significant deviation in either the rate or mode of application to the test crop from the intended use pattern?

B. Analyses to Determine Total Terminal Residues

1. Total Accountability of Radioactive Residue in Test Crop

Did the petitioner/registrant determine total recovered (i.e., combustible) radioactivity remaining in the plant at time of sampling or harvest?

For the analysis of total recovered radioactive residues, were all plant parts sampled including those which may be

processed into food or feed items at time of harvest or at a stage of development when normally utilized for animal feed, subjected to oxidative combustion analysis and subsequent Liquid Scintillation Counting of the resultant $^{14}CO_2$ formed?

Were experimental details of sample harvest and preparation provided prior to oxidative combustion analysis (i.e., dissection, grinding, lyophilization)?

If samples were not combusted as soon as possible after preparation, how long were samples stored and under what conditions? If stored, did the petitioner/registrant adequately describe sample collection, preparation and storage conditions to enable the reviewer to evaluate sample integrity?

As a result of oxidative combustion analyses of radiolabeled components in <u>all</u> plant parts and fractions examined, did the petitioner/registrant account for a majority of the total radioactivity in or on the test crop at time of sampling or harvest?

Did the petitioner/registrant determine what percentage of total recovered radioactivity was determined by subsequently developed enforcement methodology? [<u>See</u> preceding Note under II]

For <u>each sample analyzed (plant part or sample fraction)</u>, was total radioactivity reported as:

(1) total radioactive counts, (2) percentage of the total recovered plant radioactivity at the time of sampling or harvest, and (3) ppm (expressed as equivalents of parent compound) of total recovered plant radioactivity at the time of sampling or harvest? If not, then the petitioner/registrant must recalculate and resubmit his radiolabeled residue data in this format.

2. <u>Characterization/Identification of Extractable Radioactivity</u>

Did the petitioner/registrant describe the conditions employed for the acidic, basic and/or enzymatic hydrolysis of the filter cake or residue remaining from previously extracted plant tissue and/or water soluble plant extracts to facilitate release of conjugated residues from these samples? If so, was the ratio and/or amounts of total free vs conjugated parent compound and/or metabolite reported in each extracted sample matrix?

Did the petitioner/registrant utilize appropriate solvents/extraction techniques to quantitatively recover radiolabeled moieties from all sample matrices? For example, for complex bound radiolabeled matrices were exhaustive sequential Soxhlet extractions with a series of both nonpolar and polar solvents employed including additional extractions with decomplexing reagents (i.e., Na_2-EDTA or mild detergents), rather than simple extractions with or partitioning into nonpolar organic solvents which may not have captured all polar radiolabeled moieties present?

Did the petitioner/registrant provide a quantitative estimate of residual radioactivity (i.e., reported as "nonextractable, unidentified or bound") remaining in the extracted sample matrix following exhaustive solvent extractions? If so, see item (3) below for procedures to characterize these residues and assess their significance.

[Note: In many instances, the petitioner/registrant may report significant levels of "nonextractable, unidentified or bound" residues in the RAC examined, however, these residues do not reflect "bound residues" in the context of the IUPAC (1981) definition cited in Appendix 2, but in reality represent conjugated or complex bound radiolabeled residues not extractable following the inefficient or incomplete sample treatment/extraction procedures employed by the investigator.]

Overall, did the petitioner/registrant clearly demonstrate and report that the extraction protocols utilized in the plant metabolism study would have released essentially all of the conjugated or bound forms of the metabolites formed, and were radiochemical extraction efficiencies for all harvested plant tissues determined and reported?

Were adequate protocols used and results reported for fractionation and isolation of the radioactivity extracted from the sample matrices? For example, were techniques such as solvent partitioning, high voltage electrophoresis, ion-exchange column chromatography, HPLC using gradient elution with a radio-active detector and thin layer radioautography employed?

During sample fractionation, was total radioactivity monitored and reported in representative sample fractions isolated and was any loss of radioactivity accounted for?

In the reviewer's judgment were all major metabolite components (i.e., those generally comprising $\geq$ 10 percent of total recovered radioactive residue) ultimately separated and purified to the extent possible in preparative radio-HPLC fractions and in radio-TLC spots or zones to permit subsequent low-level characterization/identification by the determinative methods employed?

Were the isolated and purified metabolites characterized via Rf values by a least two different solvent systems on TLC run as a single or two-dimensional chromatogram, and by their relative retention times on HPLC and GC columns eluted with different solvents or employing various column packings and equipped with radioactive detectors? Were all known or suspected metabolites of the parent compound synthesized and cochromatographed on the same TLC plates and on the same HPLC or GC columns to facilitate in the identification of unknown sample metabolites?

If available, did the petitioner/registrant employ mass spectral analyses in the CI and/or EI modes coupled with the same HPLC or GC systems employed for the separation and characterization of metabolites described above for ultimate metabolite identification? If mass spectral analyses were employed were scans in the CI and/or EI modes obtained for all known and suspected metabolites of the parent compound and compared to those obtained for the sample metabolites to insure identification?

At a minimum, did the petitioner/registrant attempt to classify or categorize, based on the evidence at hand, all discrete extractable radiolabeled entities which could not be characterized via the chromatographic techniques employed? In the aggregate and individually what percentage of the total recovered plant radiolabeled residue and ppm (expressed as equivalents of parent compound) did these unidentified and/or uncharacterized entities represent?

If these aforementioned unidentified/uncharacterized radiolabeled entities represent a significant portion of the total recovered radiolabeled residue in plants then TB should be given as much information as is available about these entities and asked whether their concern warrants identification or whether partial characterization will suffice.

Did the petitioner/registrant identify all major metabolite components where each generally comprised $\geq$ 10 percent of the total recovered radiolabeled residue accounted for in the submitted plant metabolism study? If so, then TB should be asked whether these metabolites are to be included in the tolerance expression.

Did the petitioner/registrant, if possible, provide information on the chemical nature of discrete (minor) metabolite components?

Were individual free minor metabolite components each generally comprising < 10 percent of the total recovered radiolabeled residue <u>also</u> identified as extractable/ bioavailable and pesticidally derived (exocon) components of the bound residues characterized under Item (3) below? If so, and if their <u>total</u> radioactive concentration (free + bound) generally comprised $\geq$ 10 percent of the total recovered radioactive residue accounted for in the submitted plant metabolism study then those minor metabolite

components must also be identified. [Notes:(1) In the examples given above, the percentages provided are merely guidelines to be used by the Agency's data reviewers and are modified upward or downward based on the importance of the treated crop as a food or feed and on the toxicity of the minor metabolite component. (2) The requirements of this paragraph are not applicable to minor metabolite components which have been irreversibly converted to complex bound residues which are subsequently released as recycled radiolabeled (endocon) fragments, of no toxicological concern, following hydrolytic characterization procedures.]

At a minimum, did the petitioner/registrant provide adequate representations of radio-TLC plates and samples of HPLC/GC chromatograms as well as mass spectral scans, including a description of all equipment (and operating parameters) used for the extraction, fractionation, characterization, and identification of radiolabeled residues?

For each characterized/identified residue component (metabolite) did the petitioner/registrant report its concentration both on a ppm (expressed as equivalents of parent compound) basis and as a percentage of: (1) the total recovered radioactivity in the treated plant at harvest or when utilized as an animal feed, and (2) the total radioactivity recovered from the particular plant part or sample fraction analyzed? If not, then the petitioner/registrant must recalculate and resubmit his metabolite data in this format.

3. Characterization and Regulatory Significance of Nonextractable or Bound Residues

Were the "unidentified, nonextractable or bound" residues reported in item (2) above by the petitioner/registrant located in edible plant parts used for food or animal feed? If not, and provided that bound residues are also not expected in these same food or feed items, then no further regulatory action is required.

If "bound" residues were found in these plant parts, did they generally comprise less than 10 percent or when normalized to use conditions less than 0.1 ppm (expressed as equivalents of parent compound) of the total radioactivity recovered from the treated plant? If so, and furthermore provided that in response to RCB's deference/reference(s) (see item III (D) of this SEP) TB expresses no concern regarding the presence of these low levels of uncharacterized bound residues then no further regulatory action is required. However, if TB does express concern over the presence of these latter residues or if bound residues generally exceed 10 percent or 0.1 ppm then these residues must be further characterized by the petitioner/registrant to enable the Agency to determine their regulatory significance.

[Note: These percentage or residue levels are merely guidelines to be used by the Agency's data reviewers and are modified upward or downward based on the importance of the treated

crop as a food or feed and the toxicity of the extrac able/ bioavailable and pesticidally derived (exocon) components of the bound residue.]

When significant levels of nonextractable or bound residues were present [i.e., generally greater than 10 percent or when normalized to use conditions greater than 0.1 ppm (expressed as equivalents of parent compound) of the total radioactivity recovered from the treated plant] were bona fide attempts made by the petitioner/registrant to further characterize these residues?

Were essentially all of the conjugated or complex bound chemical species originating from the parent pesticide (exocons) extracted from the bound residue and chemically characterized?

During the course of the radiolabeled plant metabolism study, did the petitioner/registrant conduct interval sampling of protein, starch, lignin, and cellulose fractions which would indicate if significant levels of unidentified radioactivity are entering those fractions? The foregoing observations would indicate that the bound residue does not consist of discrete moieties (exocons) originating from the parent pesticide (and possibly of toxicological significance) but rather of recycled radiolabeled fragments that have been incorporated into natural products (endocons) of the plant and therefore are clearly of no toxicological concern.

[Note: The publication by M. Kovacs (1986) in Appendix 2, should be consulted regarding the characterization and fractionation procedures for plant natural products (endocons).]

As a result of these fractionation and characterization procedures, did the petitioner/registrant clearly demonstrate that the bound residues consisted primarily of recycled radiolabeled fragments that were incorporated into natural plant products? If so, then no further regulatory action is required; if not, then the exocon components of the bound residue must be characterized.

In response to RCB's deference/reference(s) to TB (see item III (D) of this SEP) were the extractable and characterized exocon components of the bound residue judged to be of toxicological concern? If not, then no further regulatory action is required; if so, then the petitioner/registrant must develop and validate modified enforcement analytical methodology to extract and detect these bound exocon components of toxicological concern.

[Note: The data reviewer is also advised to consult the publication by M. Kovacs (1986) cited in Appendix 2, concerning further details on bound residue classification, nomenclature, and characterization strategies, including a proposed tabular sequential testing approach for determining the regulatory significance of bound pesticide residues.]

C. Residue Methodology and Instrumentation

Refer to the SEP on Analytical Methods for guidance on this subject area.

D. Quality Control

Refer to the SEP on Magnitude of the Residue: Crop Field Trials, Appendix 1, Item (E)(4) Quality Control for guidance on this subject area.

E. Evaluation and Reporting Requirements

In the data reviewer's judgment, were all components of the total terminal residue, whether free or bound/conjugated characterized to the extent necessary to permit the Agency to conclude that as a result of the proposed use that the nature of the total terminal residue has been adequately characterized or delineated in the subject RAC?

Were all components of the total terminal residue, whether free or bound/conjugated characterized to the extent that TB could render an opinion regarding the toxicological significance of each major component and thereby allow the Agency to define the total toxic residue?

Did the petitioner/registrant delineate, preferably in a flowsheet, the possible routes of degradation or pathways of metabolism in the subject RAC?

When enforcement analytical methodology was developed did the petitioner/registrant validate these methods with radiolabeled samples derived from the plant metabolism study and provide a statement as to their capability to determine all components of the total toxic residue whether free or bound/conjugated? [See preceding Note under II; Also Note: The publication by M. Kovacs and C. Trichilo (1987) in Appendix 2, should be consulted for a discussion of procedures to integrate the conduct of radiolabeled plant metabolism studies with the subsequent development and validation of proposed analytical enforcement methodologies]

APPENDIX 2

REVIEWER AIDS MATERIALS

Following is a listing of some of the more useful reference citations within the Residue Chemistry Branch that could prove helpful in reviewing plant metabolism studies:

(1) Dorough, H.W., Biological activity of pesticide conjugates. In: Kaufman, D., Still, G., Paulson, G., Bandal, S. (eds) Bound and conjugated pesticide residues. Washington, D.C.; American Chemical Society Symposium Series 29: 10-34, (1976);

(2) Dorough, H.W., Classification of radioactive pesticide residues in food-producing animals. J. Environ. Path and Tox 3: 11-19, (1980);

(3) Hazard Evaluation Division, Standard Evaluation Procedure, Analytical Methods (1988);

(4) Hazard Evaluation Division, Standard Evaluation Procedure, Animal Metabolism (1988);

(5) Hazard Evaluation Division, Standard Evaluation Procedure, Magnitude of the Residue: Crop Field Trials (1985);

(6) International Union of Pure and Applied Chemistry (IUPAC) 1981 definition of bound residues in plants: 'Non-extractable residues (sometimes referred to as "bound" or "non-extracted" residues) in plants are defined as chemical species originating from pesticides, used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues. These non-extractable residues are considered to exclude fragments recycled through pathways leading to natural products.

Chemical species in this context refers either to the parent material or to derivatives or fragments of it.

Methods in this context refer to any procedures, such as solvent extraction and distillation, used to exhaustively remove chemical species from a plant matrix. In each reference to a non-extractable residue, the extraction procedure must be given.';

(7) Kaufman, D., Still, G., Paulson, G., Bandal, S. (eds) Bound and conjugated pesticide residues, Washington, D.C.; American Chemical Society Symposium Series 29:(1976);

(8) Khan SU., Bound pesticide residues in soil and plants. Residue Reviews 84: 1-25, (1982);

(9) Klein, W., Scheunert I., Bound pesticide residues in soil, plants, and food with particular emphasis on the application of nuclear techniques. In: Agrochem: Fate Food Environ., Proc. Int. Symp. IAEA, Vienna, Austria : pp.177-205, (1982);

(10) Kovacs, M.F., Regulatory aspects of bound residues (chemistry). Residue Reviews 97: 1-17, (1986);

(11) Kovacs, M.F., Trichilo, C.L., Regulatory perspective of pesticide analytical enforcement methodology in the United States. J.Assoc.Off.Anal.Chem. 70: No.6 937-940 (1987);

(12) Menzie, C.M., Metabolism of Pesticides (including three updates) Special Scientific Reports, United States Department of the Interior, Fish and Wildlife Service;

(13) Paulson, G., Caldwell, J., Hutson, D., Menn, J. (eds) Xenobiotic conjugation chemistry. Washington, D.C.; American Chemical Society Symposium Series 299: (1986);

(14) Pesticide Assessment Guidelines, Subdivision O, Addendum 3 Nature of the Residue: Plants prepared by OPTS/EPA Washington, D.C. (1987);

(15) Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, § 171-4(a)(1)and(2), prepared by OPTS/EPA, Washington, D.C. (1982);

(16) Registration Standards on various individual pesticides, prepared by OPTS/EPA (several issued each fiscal year);

(17) Sandermann, J.H., Metabolism of environmental chemicals by plants - copolymerization into lignin. In: J. Appl. Poly. Sci: Applied Polymer Symposium 37. John Wiley & Sons, Inc., New York, pp. 407-420 (1983).